Document Number 4

Entry 4 of 10 File: USPT Jun 17, 1980

DOCUMENT-IDENTIFIER: US 4208482 A
TITLE: Immobilization of glucose isomerase

BSPR:
This invention relates to immobilized enzymes, a process for preparing such immobilized enzymes, and the use of such immobilized enzymes to convert glucose to fructose in a continuous column process.

BSPR:
In answer to these problems, the art has developed immobilized enzymes, in which the enzymes are bound to inert or insoluble carriers and these immobilized enzymes can be used in columns to produce continuous reactions in which conversion of glucose to fructose takes place continuously in the column.

BSPR:
In certain cases it is advantageous to use an immobilized enzyme rather than a soluble one. The advantages of using an immobilized enzyme in comparison with one in soluble form are that the immobilized enzyme is re-usable and does not contaminate the reaction products and, therefore, is eminently suitable for continuous or repeated use. The disadvantages of the known immobilized enzymes are that some have to be prepared by rather complicated methods and, accordingly, are relatively expensive, and others require a rather large proportion of a carrier or a binder. Also some of the known immobilized enzymes suffer from the disadvantage that when packed in a column they exhibit flow properties which are not satisfactory. Moreover, a great many of the known binders are synthetic polymer products and consequently, are not suitable for production of foodstuffs.

BSPR:
In preparing the product of this invention, whole cell enzyme is entrapped in a natural hydrocolloid, specifically agar, to produce generally spherical agar gel particles which can be advantageously used in a continuous column reactor system. The whole cell enzyme may be derived from organisms of the Actinoplanes genus, preferably from Actinoplanes missouriensis or other organisms. The entrapment process of this invention is a simple one. Variations of this process render it possible to retain most of the original enzyme activity. Other known methods of immobilization of glucose isomerase often result in significant losses in enzyme activity.

DEPR:
One gram of commercial USP agar was dissolved in 25 ml of boiling water with agitation. The agar solution was then cooled and maintained at 53.degree. C. One gram of dry whole cells of Actinoplanes missouriensis NRRL B-3342 was suspended in 25 ml water at ambient temperature, then added to the agar solution and mixed. The pH of this mixture ranged from 7.0 to 7.5. Spherical particles were then formed by injecting the warm mixture into a solvent mixture of ethyl acetate and ethylene dichloride. The solvent mixture

comprised 3 parts ethyl acetate and 1 part ethylene dichloride, and the mixture was maintained at a temperature of 10.degree. C. to 15.degree. C. After decanting the solvent the spherical particles were washed with 500 ml of water. The beads were then dried to a moisture content of 6.9% (w/w) and assayed at 1,171 IGIU/g..sup.2

ORPL:
Nilsson et al., The Use of Bead Polymerization of Acrylic Monomers for Immobilization of Enzymes Biochimica et. Biophysics Actz, vol. 268, 1972 (pp. 253-256).

Document Number 2

Entry 2 of 4 File: USPT Nov 16, 1993

DOCUMENT-IDENTIFIER: US 5262313 A
TITLE: Carrageeman-immobilized esterase

BSPR:
The method of enzyme immobilization comprises several steps. A carrageenan solution is prepared and mixed with an enzyme such as an esterase, and in preferred practice, with pig liver esterase. Generally .kappa.-carrageenan is preferred for immobilization, but other carrageenans, for example, .lambda.-carrageenans may be used, although these tend to be less soluble than .kappa.-carrageenan. If heating is required to solubilize the carrageenan, the possibility of heat denaturation of the enzyme should be taken into account. Once the carrageenan and the enzyme are mixed, and before gelation has taken place, the solution may be formed into conveniently sized beads or particles. Alternatively, the solution may be allowed to gel spontaneously in the form of blocks or sheets. When beads are desired, small, generally round beads may be obtained by extruding the mixture from a needle tip and dropping into an immiscible solvent, usually an organic alcohol such as n-butanol, sec-butanol, crotyl alcohol, benzyl alcohol, isopropanol or n-propanol. If the immobilized enzyme is to be used in a hydrolysis reaction, secondary or tertiary alcohols, particularly sec-butanol, are preferred solvents in order to avoid transesterification reactions.